Claudins in viral infection: From entry to spread

Che C. Colpitts[1,2,*] and Thomas F. Baumert[1,2,3,*]

[1]Inserm, U1110, Institut de Recherche sur les Maladies Virales et Hépatiques, 67000 Strasbourg, France ; [2]Université de Strasbourg, 67000 Strasbourg, France; [3]Institut Hospitalo-Universitaire, Pôle Hépato-digestif, Hopitaux Universitaires de Strasbourg, 67000 Strasbourg, France.

***Corresponding authors**: Prof. Thomas F. Baumert, MD and Dr. Che C. Colpitts, PhD; Inserm U1110, 3 Rue Koeberlé, 67000 Strasbourg, France; Phone: ++33 3 68 85 37 03, Fax: ++33 3 68 85 37 24, e-mails: thomas.baumert@unistra.fr and colpitts@unistra.fr

Conflict of interest: the authors declare no conflict of interest. Thomas F. Baumert is a co-inventor on patent applications for claudin-1-targeting antibodies for prevention and treatment of HCV infection and liver disease

Word count: Abstract – 167; Text (excluding references and figure legends) – 2846 words; References – 106

Keywords: Claudins, tight junction, viral infection, viral pathogenesis

This article is published as part of the Special Issue on Physiology, Pathophysiology and Clinical Impact of Claudins

Abstract

Tight junctions are critically important for many physiological functions, including the maintenance of cell polarity, regulation of paracellular permeability and involvement in signal transduction pathways to regulate integral cellular processes. Furthermore, tight junctions enable epithelial cells to form physical barriers, which act as an innate immune mechanism that can impede viral infection. Viruses, in turn, have evolved mechanisms to exploit tight junction proteins to gain access to cells or spread through tissues in an infected host. Claudin family proteins are integral components of tight junctions, and are thought to play crucial roles in regulating their permeability. Claudins have been implicated in the infection process of several medically important human pathogens, including hepatitis C virus, dengue virus, West Nile virus and human immunodeficiency virus, among others. In this review, we summarize the role of claudins in viral infections, and discuss their potential as novel antiviral targets. A better understanding of claudins during viral infection may provide insight into physiological roles of claudins and uncover novel therapeutic antiviral strategies.

Introduction

Tight junctions are highly specialized membrane domains that regulate paracellular permeability by forming intercellular barriers between epithelial cells. They also provide a physical barrier between apical and basolateral membrane domains of polarized epithelial cells, thus regulating compartmentalization of membrane molecules. As such, tight junctions maintain cell polarity [84] and have also been implicated in signal transduction pathways [36].

Integral membrane tight junction proteins include occludin (OCLN) [32], claudins (CLDNs) [31] and junctional adhesion molecule-1 (JAM-1) [63]. The claudin family is thought to be the most crucial for mediating formation and permeability of tight junctions. Structurally, claudins are 25-27 kDa proteins comprised of intracellular N-terminal and C-terminal tails, four transmembrane helices, two extracellular loops (large ECL1 and small ECL2) and one cytoplasmic loop **(Figure 1)**. Typically, claudins have a signature sequence within ECL1 and a conserved C-terminal motif for binding to PDZ (PSD95, postsynaptic density protein; Dlg1, Drosophila disc large tumor suppressor; ZO-1, zonula occludens-1 protein) domains, enabling interaction with tight junction-associated scaffolding and adaptor proteins [49]. Homophilic and heterophilic interactions between different claudins as well as other TJ members mediate paracellular tightening at tight junctions, thus dictating permeability.

The mammalian claudin family is comprised of 27 different members to date, although only 26 of these are found in humans [39]. Splice variants have been reported for some of these claudins, resulting in different protein isoforms with distinct expression patterns and function. Claudins are expressed in all epithelial tissues, although claudin family members have differential expression in different

tissues [39]. Although claudins are typically found in tight junctions, non-junctional localization of some claudins has been reported [37, 48, 78]. For example, CLDN1 associates with tetraspanins on the hepatocyte cell surface to form tetraspanin enriched microdomain (TEM) [78]. Furthermore, CLDN1 directly associates with tetraspanin CD9 on Madin-Darby canine kidney cells and various carcinoma cells, and these CLDN1-CD9 complexes did not reside in tight junctions [52]. However, the physiological role of non-junctional claudin complexes remains to be identified.

Tight junctions enable epithelial cells to form tight physical barriers that help to defend against infection. Some viruses counter this by infecting epithelial cells via receptors on the apical surface. Other viruses have evolved mechanisms to bypass epithelial cell barriers, either by using tight junction proteins as entry factors or by opening the tight junctions to facilitate their entry and dissemination. Indeed, tight junctions have been implicated in the infection process of several viruses **(Table 1)**. In this review, we discuss the role of integral tight junction components, claudins, in viral infection and pathogenesis, and explore the potential of claudins as a target for antiviral therapy. Further studies into the role of claudins and tight junctions during viral infection will be important to better understand the mechanisms involved and to identify novel antiviral targets.

Claudins and viral entry

Hepatitis C virus

Claudins were first recognized to play a role in viral entry in 2007, when CLDN1 was identified as a host factor for the hepatitis C virus (HCV) [25]. HCV, a positive sense single stranded RNA virus belonging to the *Flaviviridae* family, chronically infects approximately 130 million people worldwide. These individuals are at increased risk

for severe liver disease, such as cirrhosis and hepatocellular carcinoma. As an RNA virus, HCV exists as a highly variable population of quasispecies and is classified into seven major genotypes. HCV infects hepatocytes via a complex, multi-step process involving several host proteins, including glycosaminoglycans [8, 9, 57, 83], low density lipoprotein receptor [2], very-low-density lipoprotein receptor [95], tetraspanins CD81 [76] and CD63 [74], scavenger receptor class B type 1 [82, 103], tight junction proteins CLDN1 [25] and occludin [77], E-cadherin [58], receptor tyrosine kinases such as epidermal growth factor receptor (EGFR) [59, 106], serum response factor binding protein 1 [35], cholesterol transporter Niemann-Pick C1-like 1 [80] and transferrin receptor 1 [62]. Following virion internalization [26, 68], fusion is triggered by low pH in the endosome [56]. The 9.6-kilobase genome is translated into a polyprotein of approximately 3000 amino acids [18], which then undergoes cleavage by cellular and viral proteases to generate 3 structural and 7 non-structural viral proteins.

CLDN1 plays a central role in the HCV entry process [25]. It was identified as an HCV entry factor using an iterative expression cloning approach in which non-hepatic 293T cells were transfected with a cDNA library derived from Huh7.5 hepatoma cells [25]. CLDN1 was later shown to be important for cell-to-cell transmission of HCV [12], as well as cell-free entry. Mechanistically, CLDN1 is thought to form a co-receptor complex with CD81, an interaction that likely occurs on the basolateral hepatocyte surface and involves non-TJ CLDN1 [43, 44]. The CD81-CLDN1 interaction involves the N-terminal portion of the ECL1 of CLDN1 [25]. Nine residues within the ECL1 of CLDN1 (W_{30}-I_{32}-D_{38}-EGLW_{51}-C_{54}-C_{64}) contributed to HCV entry [19, 25, 101]. Interestingly, these residues include W-GLW-C-C, the signature sequence shared by the majority of claudin family members [40]. In

particular, residues W_{30}-I_{32}-D_{38}-EG_{49}-W_{51} interacted with CD81 to promote HCV entry [20]. Formation of the CD81-CLDN1 co-receptor complex is regulated by EGFR [59], which recruits the GTPase Harvey rat sarcoma viral oncogene homolog (HRas) to mediate co-receptor interaction and downstream MAPK signaling [106].

CLDN1 may also contribute to HCV entry by direct interaction with the HCV particle. HCV envelope glycoproteins E1 and E2 coimmunoprecipitate with CLDN1 [101], and the E1E2 heterodimer was shown to recognize CLDN1 [23]. Furthermore, a genetic interaction between E1 and CLDN family members was recently described [47]. Given that HCV E2 interacts with CD81 [46, 75] [51], both HCV envelope proteins may mediate binding to the co-receptor complex.

CLDN6 and CLDN9 show high sequence homology to CLDN1 in the CD81-interacting region, and have been proposed to function as complementary HCV co-receptors [67] [104]. Although CLDN6 and CLDN9 are predominantly expressed in non-hepatic epithelial tissue during embryonic or neonate development [1, 69, 70, 87, 94], their expression could be detected in human hepatoma cell lines, primary human hepatocytes and liver tissue by qPCR approaches [67, 104]. Expression of CLDN6 and CLDN9 in CLDN-deficient 293T cells facilitated entry of HCV pseudoparticles (HCVpp) and cell culture-derived HCV (HCVcc) [67, 104]. Furthermore, CLDN9 expression in CLDN1-deficient Bel7402 human hepatoma cells permitted HCV entry [104]. However, CLDN receptor usage appears to be genotype-dependent [41]. Genotypes 2, 5 and 7 were restricted to CLDN1, whereas other genotypes were able to efficiently use CLDN1 or CLDN6 for entry, as was shown in a CLDN-deficient cell line [41]. Interesting, strong selection pressure led to a single amino acid substitution in the E1 protein of a CLDN1-dependent strain of HCV (genotype 2), conferring the ability to enter hepatocytes via CLDN6 [47].

As the CD81-CLDN1 co-receptor complex is essential for HCV entry, but lacks any known physiological (or pathological) function, therapies directed towards CLDN1 show great promise in preclinical models. Peptides derived from CLDN1 sequences inhibit HCV entry in cell culture models [85]. Monoclonal antibodies that target CLDN1 interfere with the formation of the co-receptor complex [54]. These antibodies pan-genotypically inhibit cell-free infection and cell-to-cell transmission of HCV in human hepatoma cells [27, 54, 100]. Strikingly, anti-CLDN1 antibodies prevent HCV infection of human liver chimeric mice [30, 60] and were even able to cure chronically HCV-infected liver chimeric mice [60] without any detectable toxicity.

One possible limitation for CLDN1-directed therapies is escape via CLDN6 or CLDN9 on hepatocytes. Indeed, CLDN1-specific antibodies only partially inhibited infection by HCV strains with broad CLDN tropism in CLDN6-expressing Huh7-derived hepatoma cell lines [41]. However, the functional relevance of these findings is not clear. CLDN6 and CLDN9 expression could not be detected at the protein level in primary human hepatocytes [67], and CLDN6 protein expression could not be detected in liver sections [28]. Furthermore, anti-CLDN6 and anti-CLDN9 antibodies did not inhibit HCV infection of hepatoma cells or primary hepatocytes [28]. Most importantly, treatment with an anti-CLDN1 monoclonal antibody cured chronically infected human liver chimeric mice, without escape [60]. Overall, these findings suggest that low expression levels of CLDN6 and CLDN9 likely precludes their use as HCV entry factors, at least in the majority of patients.

Dengue virus

Like HCV, Dengue virus (DENV) is a positive sense single stranded RNA virus in the *Flaviviridae* family. The DENV genome is ~10.7-kb in length, encoding for 3

structural proteins (capsid, C; premembrane, prM; envelope, E) and 7 non-structural proteins [34]. Although DENV does not cause chronic infection, it is nonetheless a serious cause of morbidity and mortality around the world, particularly as it is a mosquito-borne virus endemic in over 100 countries [10]. In some patients, dengue virus infection leads to severe dengue hemorrhagic fever and shock syndrome (DHF/DSS) [55]. No specific antiviral therapy or vaccine is yet available.

The molecular mechanisms of DENV entry into human cells remain unclear. Several host proteins have been proposed as DENV entry factors, including glycosminoglycans, lectins, glycosphingolipids and laminin-binding proteins [45]. Among these potential factors, CLDN1 was shown to be involved in DENV entry [17, 33]. CLDN1 knockout studies and RNAi-mediated gene silencing in hepatoma cells inhibited DENV infection. The DENV prM protein binds to CLDN1 residues I_{32}, C_{54} and C_{64}, which interestingly are also implicated in HCV infection [17]. Further studies are needed to elucidate the mechanistic role of CLDN1 during DENV entry and potential roles of other CLDN family members.

Claudins and viral spread

Viral infection and dissemination within a host is limited by epithelial cells, which line surfaces of organs and tissues to form a physical barrier enforced by tight junctions. Therefore, disruption of tight junctions in epithelial and endothelial barriers facilitates viral dissemination within the infected host. Several viruses, therefore, modulate expression of tight junction proteins such as claudins to enhance their dissemination.

West Nile virus

Like HCV and DENV, West Nile virus is classified in the *Flaviviridae* family and has a single-stranded positive sense RNA genome of ~11 kb [13]. WNV is a mosquito-borne virus capable of infecting the central nervous system, resulting in neurological disease in a minority of infected patients. Following mosquito transmission, the virus crosses several polarized cell layers, requiring a disruption of tight junctions by mechanisms that are not completely understood. One study in kidney tubules reported that WNV capsid protein induced the degradation of CLDN1, CLDN2, CLDN3 and CLDN4 by lysosomal proteases [66]. In endothelial cells, the disruption of tight junctions was found to be an indirect result of WNV infection, by secretion of matrix metalloproteases from infected astrocytes [96, 97]. Furthermore, matrix metalloprotease 9 was found to be involved in WNV infection of the brain by disrupting the blood brain barrier [98]. More recently, Xu and colleagues reported that CLDN1 and other tight junction proteins undergo endocytosis in WNV-infected endothelial and epithelial cells, resulting in the lysosomal degradation of tight junction proteins [99]. Interestingly, CLDN1 degradation was also observed in endothelial and epithelial cells infected with Japanese encephalitis virus (a related neurotropic flavivirus) [3], but not DENV [99]. For WNV, multiple mechanisms of tight junction disruption appear to be involved, ranging from WNV-induced endocytosis and degradation of tight junction proteins to immune cell-mediated production of matrix metalloproteases and proinflammatory cytokines.

Human immunodeficiency virus

Human immunodeficiency virus 1 (HIV-1) belongs to the *Retroviridae* family. HIV-1 has a single stranded positive sense RNA genome, which undergoes reverse transcription into double stranded DNA that is integrated into the host genome. Thus,

HIV establishes a persistent, life-long infection. HIV-1 is transmitted via sexual routes, which requires the virus to cross genital or intestinal epithelial cell layers. HIV-1 typically infects CD4+ T cells, via interaction between viral gp120 and CD4 [65]. Interestingly, one study reported that HIV-1 is capable of using CLDN7 as an entry factor in CD4-negative T cells independently of gp120 [105], although the physiological relevance remains unclear. To facilitate viral dissemination, HIV-1 has been shown to alter the expression of tight junction claudins in intestinal cell lines and primary genital epithelial cells [24, 72, 93]. HIV-1 infection can also lead to neuropathogenesis [11], following invasion of the central nervous system. Indeed, HIV-1 disrupts the tight junctions of the blood brain barrier [89], at least in part by downregulating CLDN5 expression [16], which may be the result of virus-induced post-translational modifications of CLDN5 [7]. This effect may be mediated by the viral Tat protein, which was shown to alter CLDN1 and CLDN5 expression in brain endothelial cells and in the brains of mice [4, 5]. In colonic cell lines and primary genital epithelial cells, gp120 was shown to disrupt CLDN1, CLDN2 and CLDN4 expression and increase permeability [72]. Other tight junction proteins were also implicated [4]. Interestingly, disruption of tight junctions by HIV proteins was found to facilitate the paracellular spread of herpes simplex virus type 1 and type 2 [86].

Respiratory viruses

Human rhinovirus (HRV), a single stranded positive sense RNA virus in the *Picornaviridae* family, is the main cause of the common cold and as such is one of the most common viral infections in humans [50]. Respiratory syncytial virus (RSV) is a single stranded negative sense RNA virus in the *Paramyxoviridae* family. It is a common cause of lower respiratory tract infections, particularly in children and immunocompromised individuals [42]. To establish infection, these viruses must

bypass the airway epithelium, a well-developed barrier regulated by tight junctions. Therefore, respiratory viruses have evolved mechanisms to disrupt or alter tight junctions. In primary human nasal epithelial cells, HRV was shown to decrease expression of CLDN1 and other tight junction components, thus increasing permeability [102]. Similarly, RSV decreased expression of CLDN1 in primary human nasal epithelial cells [64]. Conversely, however, RSV infection induced expression of CLDN4 and other tight junction components, which was suggested to facilitate viral assembly and budding at the apical membrane by promoting cell polarity [64].

Gastrointestinal viruses

Rotavirus (RV), a double stranded RNA virus in the *Reoviridae* family, infects the gastrointestinal tract and is a major causative agent of diarrhea in children [21]. RV infection of intestinal epithelial cell lines altered the distribution of CLDN1 and CLDN3, thus disrupting tight junctions and resulting in decreased permeability [22, 71]. This effect was mediated at least in part by viral protein VP8 [71]. Given that RV uses integrins for cell entry [38], this disruption of the tight junctions was proposed to open paracellular spaces, allowing access to the receptors. This likely contributes to pathogenesis by disrupting the intestinal lining.

Norovirus (NV), a single stranded positive sense RNA virus in the Caliciviridae family, is the most common cause of viral gastroenteritis [79]. As for RV, infection with NV leads to a reduction in expression of tight junction proteins, including CLDN4 and CLDN5 [92]. The resulting epithelial barrier dysfunction contributes to pathogenic effects of infection, such as diarrhea.

Beyond claudins: Tight junctions and viral infection

Although claudins are key players in tight junction physiology, other proteins from different families contribute to tight junction structure and function. The Junctional Adhesion Molecules (JAM-A, JAM-B and JAM-C) as well as the Coxsackievirus and Adenovirus Receptor (CAR) [29] are part of the immunoglobulin superfamily. CAR was the first tight junction protein shown to be involved in viral entry, and mediates the entry of both adenoviruses and coxsackieviruses, by different mechanisms [15]. JAM-A, another tight junction component, is important for the entry and internalization of reoviruses [14], such as RV [91], as well as feline calicivirus [61]. Indeed, viruses from several different families use tight junction proteins either for entry, dissemination or egress [90]. Further research into tight junctions with respect to viral infection will allow for a better understanding of viral replication/dissemination strategies and may provide targets for novel therapeutic strategies.

Conclusions and perspectives

As key components of tight junctions, claudins are critical for the regulation of tight junction structure and function. Given that claudins regulate paracellular permeability and form barriers between epithelial cells, several viruses evolved mechanisms to use claudins during their replication cycle or to disseminate within the host. As such, claudins are attractive antiviral targets. Indeed, antibodies and peptides targeting CLDN1 have been shown to be highly effective against HCV [27, 30, 54, 60, 73, 85, 100]. These antibodies are thought to block formation of the nonphysiological CLDN1-CD81 co-receptor complex during HCV entry, and thus lack any obvious toxic effects for the host. The potential of claudins as antiviral targets in the context of

other viral infections remains to be established. Furthermore, claudins are implicated in the pathology of several diseases. Further research into the roles of claudins in viral infection will provide insight into their physiological roles, opening avenues for novel antiviral and therapeutic strategies.

Acknowledgements: TFB acknowledges support through funding from the European Union (ERC-2014-AdG-HEPCIR, FP7 HepaMAb, H2020 HEPCAR and Interreg IV FEDER-Hepato-Regio-Net 2012), the US National Institutes of Health (1 U19 AI23862 01) the Agence Nationale de Recherches sur le SIDA (ANRS), the Direction Générale de l'Offre de Soins (A12027MS), Inserm and the University of Strasbourg Foundation. This work has been published under the framework of the LABEX ANR-10-LABX-0028_HEPSYS and benefits from funding from the state managed by the French National Research Agency as part of the Investments for the future program. CCC is supported by fellowships from the Canadian Institutes of Health Research (201411MFE- 338606-245517) and the Canadian Network on Hepatitis C.

Table 1. Claudin usage by different viruses.

Virus	Claudin member	Function	Reference(s)
Hepatitis C virus	CLDN1, CLDN6, CLDN9	Viral entry into hepatocytes, cell-to-cell transmission	[19, 25, 26, 41, 67, 104]
Dengue virus	CLDN1	Viral entry	[17, 33]
West Nile virus	CLDN1, CLDN2, CLDN3, CLDN4	Degradation of claudins facilitates viral dissemination into the brain	[66, 96, 97, 99]
Japanese encephalitis virus	CLDN1	Degradation of CLDN1 likely facilitates viral dissemination	[3]
Human immunodeficiency virus	CLDN7 CLDN1, CLDN2, CLDN4, CLDN5	Viral entry into CD4(-) T cells in gp120-independent manner Degradation of claudins facilities viral dissemination	[105] [4, 5, 7, 16, 24, 72, 93]
Human rhinovirus	CLDN1	Degradation of CLDN1 facilitates viral access to receptors	[102]
Respiratory syncytial virus	CLDN1 CLDN4	Degradation of CLDN1 facilitates viral access to receptors Induction of CLDN4 expression facilitates viral budding at apical membrane	[64] [64]
Rotavirus	CLDN1, CLDN3	Disruption of claudin localization facilitates viral access to receptors	[22, 71]
Norovirus	CLDN4, CLDN5	Reduction in claudin expression may enhance viral infection/dissemination	[92]

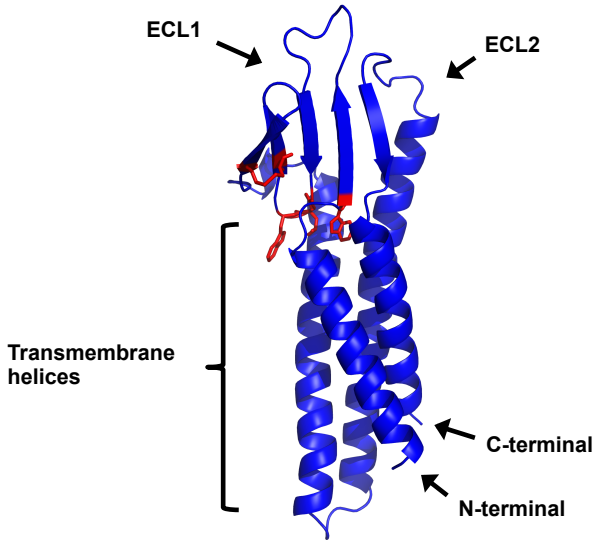

Figure 1. Homology model showing the general structure of CLDN1 as a representative claudin. Claudin signature sequence residues within ECL1 (W-GLW-C-C) are shown in red with side chains. The homology model was generated using the crystal structure of murine CLDN19 [81] as a template in SWISS-MODEL [6]. CLDN19 is closely related to CLDN1 [53]. The structure was rendered in PyMol [88]. ECL1, extracellular loop 1; ECL2, extracellular loop 2.

References:

1. Abuazza G, Becker A, Williams SS, Chakravarty S, Truong HT, Lin F, and Baum M (2006) Claudins 6, 9, and 13 are developmentally expressed renal tight junction proteins. *Am J Physiol Renal Physiol* 291: F1132-1141
2. Agnello V, Abel G, Elfahal M, Knight GB, and Zhang QX (1999) Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 96: 12766-12771
3. Agrawal T, Sharvani V, Nair D, and Medigeshi GR (2013) Japanese encephalitis virus disrupts cell-cell junctions and affects the epithelial permeability barrier functions. *PLoS One* 8: e69465
4. Andras IE, Pu H, Deli MA, Nath A, Hennig B, and Toborek M (2003) HIV-1 Tat protein alters tight junction protein expression and distribution in cultured brain endothelial cells. *J Neurosci Res* 74: 255-265
5. Andras IE, Pu H, Tian J, Deli MA, Nath A, Hennig B, and Toborek M (2005) Signaling mechanisms of HIV-1 Tat-induced alterations of claudin-5 expression in brain endothelial cells. *J Cereb Blood Flow Metab* 25: 1159-1170
6. Arnold K, Bordoli L, Kopp J, and Schwede T (2006) The Swiss-model workspace: A web-based environment for protein structure homology modelling. *Bioinformatics* 22: 195-201
7. Awan FM, Anjum S, Obaid A, Ali A, Paracha RZ, and Janjua HA (2014) In-silico analysis of claudin-5 reveals novel putative sites for post-translational modifications: Insights into potential molecular determinants of blood-brain barrier breach during HIV-1 infiltration. *Infect Genet Evol* 27: 355-365
8. Barth H, Schafer C, Adah MI, Zhang F, Linhardt RJ, Toyoda H, Kinoshita-Toyoda A, Toida T, Van Kuppevelt TH, Depla E, Von Weizsacker F, Blum HE,

and Baumert TF (2003) Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. *J Biol Chem* 278: 41003-41012

9. Barth H, Schnober EK, Zhang F, Linhardt RJ, Depla E, Boson B, Cosset FL, Patel AH, Blum HE, and Baumert TF (2006) Viral and cellular determinants of the hepatitis C virus envelope-heparan sulfate interaction. *J Virol* 80: 10579-10590
10. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GR, Simmons CP, Scott TW, Farrar JJ, and Hay SI (2013) The global distribution and burden of dengue. *Nature* 496: 504-507
11. Boisse L, Gill MJ, and Power C (2008) HIV infection of the central nervous system: Clinical features and neuropathogenesis. *Neurol Clin* 26: 799-819, x
12. Brimacombe CL, Grove J, Meredith LW, Hu K, Syder AJ, Flores MV, Timpe JM, Krieger SE, Baumert TF, Tellinghuisen TL, Wong-Staal F, Balfe P, and McKeating JA (2011) Neutralizing antibody-resistant hepatitis C virus cell-to-cell transmission. *J Virol* 85: 596-605
13. Brinton MA (2014) Replication cycle and molecular biology of the West Nile virus. *Viruses* 6: 13-53
14. Campbell JA, Schelling P, Wetzel JD, Johnson EM, Forrest JC, Wilson GA, Aurrand-Lions M, Imhof BA, Stehle T, and Dermody TS (2005) Junctional adhesion molecule a serves as a receptor for prototype and field-isolate strains of mammalian reovirus. *J Virol* 79: 7967-7978
15. Carson SD (2001) Receptor for the group B coxsackieviruses and adenoviruses: CAR. *Rev Med Virol* 11: 219-226

16. Chaudhuri A, Yang B, Gendelman HE, Persidsky Y, and Kanmogne GD (2008) STAT1 signaling modulates HIV-1-induced inflammatory responses and leukocyte transmigration across the blood-brain barrier. *Blood* 111: 2062-2072

17. Che P, Tang H, and Li Q (2013) The interaction between claudin-1 and dengue viral prM/M protein for its entry. *Virology* 446: 303-313

18. Choo QL, Richman KH, Han JH, Berger K, Lee C, Dong C, Gallegos C, Coit D, Medina-Selby R, Barr PJ, and et al. (1991) Genetic organization and diversity of the hepatitis C virus. *Proc Natl Acad Sci U S A* 88: 2451-2455

19. Cukierman L, Meertens L, Bertaux C, Kajumo F, and Dragic T (2009) Residues in a highly conserved claudin-1 motif are required for hepatitis C virus entry and mediate the formation of cell-cell contacts. *J Virol* 83: 5477-5484

20. Davis C, Harris HJ, Hu K, Drummer HE, McKeating JA, Mullins JG, and Balfe P (2012) In silico directed mutagenesis identifies the CD81/claudin-1 hepatitis C virus receptor interface. *Cell Microbiol* 14: 1892-1903

21. Desselberger U (2014) Rotaviruses. *Virus Res* 190: 75-96

22. Dickman KG, Hempson SJ, Anderson J, Lippe S, Zhao L, Burakoff R, and Shaw RD (2000) Rotavirus alters paracellular permeability and energy metabolism in Caco-2 cells. *Am J Physiol Gastrointest Liver Physiol* 279: G757-766

23. Douam F, Dao Thi VL, Maurin G, Fresquet J, Mompelat D, Zeisel MB, Baumert TF, Cosset FL, and Lavillette D (2014) Critical interaction between E1 and E2 glycoproteins determines binding and fusion properties of hepatitis C virus during cell entry. *Hepatology* 59: 776-788

24. Epple HJ, Schneider T, Troeger H, Kunkel D, Allers K, Moos V, Amasheh M, Loddenkemper C, Fromm M, Zeitz M, and Schulzke JD (2009) Impairment of

the intestinal barrier is evident in untreated but absent in suppressively treated HIV-infected patients. *Gut* 58: 220-227

25. Evans MJ, von Hahn T, Tscherne DM, Syder AJ, Panis M, Wolk B, Hatziioannou T, McKeating JA, Bieniasz PD, and Rice CM (2007) Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 446: 801-805

26. Farquhar MJ, Hu K, Harris HJ, Davis C, Brimacombe CL, Fletcher SJ, Baumert TF, Rappoport JZ, Balfe P, and McKeating JA (2012) Hepatitis C virus induces CD81 and claudin-1 endocytosis. *J Virol* 86: 4305-4316

27. Fofana I, Krieger SE, Grunert F, Glauben S, Xiao F, Fafi-Kremer S, Soulier E, Royer C, Thumann C, Mee CJ, McKeating JA, Dragic T, Pessaux P, Stoll-Keller F, Schuster C, Thompson J, and Baumert TF (2010) Monoclonal anti-claudin 1 antibodies prevent hepatitis C virus infection of primary human hepatocytes. *Gastroenterology* 139: 953-964, 964 e951-954

28. Fofana I, Zona L, Thumann C, Heydmann L, Durand SC, Lupberger J, Blum HE, Pessaux P, Gondeau C, Reynolds GM, McKeating JA, Grunert F, Thompson J, Zeisel MB, and Baumert TF (2013) Functional analysis of claudin-6 and claudin-9 as entry factors for hepatitis C virus infection of human hepatocytes by using monoclonal antibodies. *J Virol* 87: 10405-10410

29. Freimuth P, Philipson L, and Carson SD (2008) The coxsackievirus and adenovirus receptor. *Curr Top Microbiol Immunol* 323: 67-87

30. Fukasawa M, Nagase S, Shirasago Y, Iida M, Yamashita M, Endo K, Yagi K, Suzuki T, Wakita T, Hanada K, Kuniyasu H, and Kondoh M (2015) Monoclonal antibodies against extracellular domains of claudin-1 block hepatitis C virus infection in a mouse model. *J Virol* 89: 4866-4879

31. Furuse M, Fujita K, Hiiragi T, Fujimoto K, and Tsukita S (1998) Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J Cell Biol* 141: 1539-1550

32. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, and Tsukita S (1993) Occludin: A novel integral membrane protein localizing at tight junctions. *J Cell Biol* 123: 1777-1788

33. Gao F, Duan X, Lu X, Liu Y, Zheng L, Ding Z, and Li J (2010) Novel binding between pre-membrane protein and claudin-1 is required for efficient dengue virus entry. *Biochem Biophys Res Commun* 391: 952-957

34. Gebhard LG, Filomatori CV, and Gamarnik AV (2011) Functional RNA elements in the dengue virus genome. *Viruses* 3: 1739-1756

35. Gerold G, Meissner F, Bruening J, Welsch K, Perin PM, Baumert TF, Vondran FW, Kaderali L, Marcotrigiano J, Khan AG, Mann M, Rice CM, and Pietschmann T (2015) Quantitative proteomics identifies serum response factor binding protein 1 as a host factor for hepatitis C virus entry. *Cell Rep* 12: 864-878

36. Gonzalez-Mariscal L, Tapia R, and Chamorro D (2008) Crosstalk of tight junction components with signaling pathways. *Biochim Biophys Acta* 1778: 729-756

37. Gregory M, Dufresne J, Hermo L, and Cyr D (2001) Claudin-1 is not restricted to tight junctions in the rat epididymis. *Endocrinology* 142: 854-863

38. Guerrero CA, Mendez E, Zarate S, Isa P, Lopez S, and Arias CF (2000) Integrin alpha(v)beta(3) mediates rotavirus cell entry. *Proc Natl Acad Sci U S A* 97: 14644-14649

39. Gunzel D, and Fromm M (2012) Claudins and other tight junction proteins. *Compr Physiol* 2: 1819-1852

40. Gunzel D, and Yu AS (2013) Claudins and the modulation of tight junction permeability. *Physiol Rev* 93: 525-569

41. Haid S, Grethe C, Dill MT, Heim M, Kaderali L, and Pietschmann T (2014) Isolate-dependent use of claudins for cell entry by hepatitis C virus. *Hepatology* 59: 24-34

42. Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, Auinger P, Griffin MR, Poehling KA, Erdman D, Grijalva CG, Zhu Y, and Szilagyi P (2009) The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 360: 588-598

43. Harris HJ, Davis C, Mullins JG, Hu K, Goodall M, Farquhar MJ, Mee CJ, McCaffrey K, Young S, Drummer H, Balfe P, and McKeating JA (2010) Claudin association with CD81 defines hepatitis C virus entry. *J Biol Chem* 285: 21092-21102

44. Harris HJ, Farquhar MJ, Mee CJ, Davis C, Reynolds GM, Jennings A, Hu K, Yuan F, Deng H, Hubscher SG, Han JH, Balfe P, and McKeating JA (2008) CD81 and claudin 1 coreceptor association: Role in hepatitis C virus entry. *J Virol* 82: 5007-5020

45. Hidari KI, and Suzuki T (2011) Dengue virus receptor. *Trop Med Health* 39: 37-43

46. Higginbottom A, Quinn ER, Kuo CC, Flint M, Wilson LH, Bianchi E, Nicosia A, Monk PN, McKeating JA, and Levy S (2000) Identification of amino acid residues in CD81 critical for interaction with hepatitis C virus envelope glycoprotein E2. *J Virol* 74: 3642-3649

47. Hopcraft SE, and Evans MJ (2015) Selection of a hepatitis C virus with altered entry factor requirements reveals a genetic interaction between the E1 glycoprotein and claudins. *Hepatology* 62: 1059-1069

48. Inai T, Sengoku A, Hirose E, Iida H, and Shibata Y (2007) Claudin-7 expressed on lateral membrane of rat epididymal epithelium does not form aberrant tight junction strands. *Anat Rec (Hoboken)* 290: 1431-1438

49. Itoh M, Furuse M, Morita K, Kubota K, Saitou M, and Tsukita S (1999) Direct binding of three tight junction-associated MAGUKS, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J Cell Biol* 147: 1351-1363

50. Jacobs SE, Lamson DM, St George K, and Walsh TJ (2013) Human rhinoviruses. *Clin Microbiol Rev* 26: 135-162

51. Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, Dai X, Stanfield RL, Burton DR, Ward AB, Wilson IA, and Law M (2013) Hepatitis C virus E2 envelope glycoprotein core structure. *Science* 342: 1090-1094

52. Kovalenko OV, Yang XH, and Hemler ME (2007) A novel cysteine cross-linking method reveals a direct association between claudin-1 and tetraspanin CD9. *Mol Cell Proteomics* 6: 1855-1867

53. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, and Blasig IE (2008) Structure and function of claudins. *Biochim Biophys Acta* 1778: 631-645

54. Krieger SE, Zeisel MB, Davis C, Thumann C, Harris HJ, Schnober EK, Mee C, Soulier E, Royer C, Lambotin M, Grunert F, Dao Thi VL, Dreux M, Cosset FL, McKeating JA, Schuster C, and Baumert TF (2010) Inhibition of hepatitis C virus infection by anti-claudin-1 antibodies is mediated by neutralization of E2-CD81-claudin-1 associations. *Hepatology* 51: 1144-1157

55. Kularatne SA (2015) Dengue fever. *BMJ* 351: h4661

56. Lavillette D, Bartosch B, Nourrisson D, Verney G, Cosset FL, Penin F, and Pecheur EI (2006) Hepatitis C virus glycoproteins mediate low pH-dependent membrane fusion with liposomes. *J Biol Chem* 281: 3909-3917

57. Lefevre M, Felmlee DJ, Parnot M, Baumert TF, and Schuster C (2014) Syndecan 4 is involved in mediating HCV entry through interaction with lipoviral particle-associated apolipoprotein E. *PLoS One* 9: e95550

58. Li Q, Sodroski C, Lowey B, Schweitzer CJ, Cha H, Zhang F, and Liang TJ (2016) Hepatitis C virus depends on E-cadherin as an entry factor and regulates its expression in epithelial-to-mesenchymal transition. *Proc Natl Acad Sci U S A* 113: 7620-7625

59. Lupberger J, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, Davis C, Mee CJ, Turek M, Gorke S, Royer C, Fischer B, Zahid MN, Lavillette D, Fresquet J, Cosset FL, Rothenberg SM, Pietschmann T, Patel AH, Pessaux P, Doffoel M, Raffelsberger W, Poch O, McKeating JA, Brino L, and Baumert TF (2011) EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. *Nat Med* 17: 589-595

60. Mailly L, Xiao F, Lupberger J, Wilson GK, Aubert P, Duong FH, Calabrese D, Leboeuf C, Fofana I, Thumann C, Bandiera S, Lutgehetmann M, Volz T, Davis C, Harris HJ, Mee CJ, Girardi E, Chane-Woon-Ming B, Ericsson M, Fletcher N, Bartenschlager R, Pessaux P, Vercauteren K, Meuleman P, Villa P, Kaderali L, Pfeffer S, Heim MH, Neunlist M, Zeisel MB, Dandri M, McKeating JA, Robinet E, and Baumert TF (2015) Clearance of persistent hepatitis C virus infection in humanized mice using a claudin-1-targeting monoclonal antibody. *Nat Biotechnol* 33: 549-554

61. Makino A, Shimojima M, Miyazawa T, Kato K, Tohya Y, and Akashi H (2006) Junctional adhesion molecule 1 is a functional receptor for feline calicivirus. *J Virol* 80: 4482-4490

62. Martin DN, and Uprichard SL (2013) Identification of transferrin receptor 1 as a hepatitis C virus entry factor. *Proc Natl Acad Sci U S A* 110: 10777-10782

63. Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, Simmons D, and Dejana E (1998) Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol* 142: 117-127

64. Masaki T, Kojima T, Okabayashi T, Ogasawara N, Ohkuni T, Obata K, Takasawa A, Murata M, Tanaka S, Hirakawa S, Fuchimoto J, Ninomiya T, Fujii N, Tsutsumi H, Himi T, and Sawada N (2011) A nuclear factor-kappa B signaling pathway via protein kinase C delta regulates replication of respiratory syncytial virus in polarized normal human nasal epithelial cells. *Mol Biol Cell* 22: 2144-2156

65. McDougal JS, Kennedy MS, Sligh JM, Cort SP, Mawle A, and Nicholson JK (1986) Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110k viral protein and the T4 molecule. *Science* 231: 382-385

66. Medigeshi GR, Hirsch AJ, Brien JD, Uhrlaub JL, Mason PW, Wiley C, Nikolich-Zugich J, and Nelson JA (2009) West Nile virus capsid degradation of claudin proteins disrupts epithelial barrier function. *J Virol* 83: 6125-6134

67. Meertens L, Bertaux C, Cukierman L, Cormier E, Lavillette D, Cosset FL, and Dragic T (2008) The tight junction proteins claudin-1, -6, and -9 are entry cofactors for hepatitis C virus. *J Virol* 82: 3555-3560

68. Meertens L, Bertaux C, and Dragic T (2006) Hepatitis C virus entry requires a critical postinternalization step and delivery to early endosomes via clathrin-coated vesicles. *J Virol* 80: 11571-11578

69. Morita K, Furuse M, Fujimoto K, and Tsukita S (1999) Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc Natl Acad Sci U S A* 96: 511-516

70. Nakano Y, Kim SH, Kim HM, Sanneman JD, Zhang Y, Smith RJ, Marcus DC, Wangemann P, Nessler RA, and Banfi B (2009) A claudin-9-based ion permeability barrier is essential for hearing. *PLoS Genet* 5: e1000610

71. Nava P, Lopez S, Arias CF, Islas S, and Gonzalez-Mariscal L (2004) The rotavirus surface protein VP8 modulates the gate and fence function of tight junctions in epithelial cells. *J Cell Sci* 117: 5509-5519

72. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, Gray-Owen SD, Arsenault AL, and Kaushic C (2010) Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. *PLoS Pathog* 6: e1000852

73. Paciello R, Urbanowicz RA, Riccio G, Sasso E, McClure CP, Zambrano N, Ball JK, Cortese R, Nicosia A, and De Lorenzo C (2016) Novel human anti-claudin 1 mAbs inhibit hepatitis C virus infection and may synergize with anti-SRB1 mab. *J Gen Virol* 97: 82-94

74. Park JH, Park S, Yang JS, Kwon OS, Kim S, and Jang SK (2013) Discovery of cellular proteins required for the early steps of HCV infection using integrative genomics. *PLoS One* 8: e60333

75. Petracca R, Falugi F, Galli G, Norais N, Rosa D, Campagnoli S, Burgio V, Di Stasio E, Giardina B, Houghton M, Abrignani S, and Grandi G (2000)

Structure-function analysis of hepatitis C virus envelope-CD81 binding. *J Virol* 74: 4824-4830

76. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, and Abrignani S (1998) Binding of hepatitis C virus to CD81. *Science* 282: 938-941

77. Ploss A, Evans MJ, Gaysinskaya VA, Panis M, You H, de Jong YP, and Rice CM (2009) Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 457: 882-886

78. Reynolds GM, Harris HJ, Jennings A, Hu K, Grove J, Lalor PF, Adams DH, Balfe P, Hubscher SG, and McKeating JA (2008) Hepatitis C virus receptor expression in normal and diseased liver tissue. *Hepatology* 47: 418-427

79. Robilotti E, Deresinski S, and Pinsky BA (2015) Norovirus. *Clin Microbiol Rev* 28: 134-164

80. Sainz B, Jr., Barretto N, Martin DN, Hiraga N, Imamura M, Hussain S, Marsh KA, Yu X, Chayama K, Alrefai WA, and Uprichard SL (2012) Identification of the Niemann-Pick C1-like 1 cholesterol absorption receptor as a new hepatitis C virus entry factor. *Nat Med* 18: 281-285

81. Saitoh Y, Suzuki H, Tani K, Nishikawa K, Irie K, Ogura Y, Tamura A, Tsukita S, and Fujiyoshi Y (2015) Tight junctions. Structural insight into tight junction disassembly by clostridium perfringens enterotoxin. *Science* 347: 775-778

82. Scarselli E, Ansuini H, Cerino R, Roccasecca RM, Acali S, Filocamo G, Traboni C, Nicosia A, Cortese R, and Vitelli A (2002) The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 21: 5017-5025

83. Shi Q, Jiang J, and Luo G (2013) Syndecan-1 serves as the major receptor for attachment of hepatitis C virus to the surfaces of hepatocytes. *J Virol* 87: 6866-6875

84. Shin K, Fogg VC, and Margolis B (2006) Tight junctions and cell polarity. *Annu Rev Cell Dev Biol* 22: 207-235

85. Si Y, Liu S, Liu X, Jacobs JL, Cheng M, Niu Y, Jin Q, Wang T, and Yang W (2012) A human claudin-1-derived peptide inhibits hepatitis C virus entry. *Hepatology* 56: 507-515

86. Sufiawati I, and Tugizov SM (2014) HIV-associated disruption of tight and adherens junctions of oral epithelial cells facilitates HSV-1 infection and spread. *PLoS One* 9: e88803

87. Sugimoto K, Ichikawa-Tomikawa N, Satohisa S, Akashi Y, Kanai R, Saito T, Sawada N, and Chiba H (2013) The tight-junction protein claudin-6 induces epithelial differentiation from mouse F9 and embryonic stem cells. *PLoS One* 8: e75106

88. The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC

89. Toborek M, Lee YW, Flora G, Pu H, Andras IE, Wylegala E, Hennig B, and Nath A (2005) Mechanisms of the blood-brain barrier disruption in HIV-1 infection. *Cell Mol Neurobiol* 25: 181-199

90. Torres-Flores JM, and Arias CF (2015) Tight junctions go viral! *Viruses* 7: 5145-5154

91. Torres-Flores JM, Silva-Ayala D, Espinoza MA, Lopez S, and Arias CF (2015) The tight junction protein JAM-A functions as coreceptor for rotavirus entry into MA104 cells. *Virology* 475: 172-178

92. Troeger H, Loddenkemper C, Schneider T, Schreier E, Epple HJ, Zeitz M, Fromm M, and Schulzke JD (2009) Structural and functional changes of the duodenum in human norovirus infection. *Gut* 58: 1070-1077

93. Tugizov SM, Herrera R, Chin-Hong P, Veluppillai P, Greenspan D, Michael Berry J, Pilcher CD, Shiboski CH, Jay N, Rubin M, Chein A, and Palefsky JM (2013) HIV-associated disruption of mucosal epithelium facilitates paracellular penetration by human papillomavirus. *Virology* 446: 378-388

94. Turksen K, and Troy TC (2001) Claudin-6: A novel tight junction molecule is developmentally regulated in mouse embryonic epithelium. *Dev Dyn* 222: 292-300

95. Ujino S, Nishitsuji H, Hishiki T, Sugiyama K, Takaku H, and Shimotohno K (2016) Hepatitis C virus utilizes VLVDR as a novel entry pathway. *Proc Natl Acad Sci U S A* 113: 188-193

96. Verma S, Kumar M, Gurjav U, Lum S, and Nerurkar VR (2010) Reversal of West Nile virus-induced blood-brain barrier disruption and tight junction proteins degradation by matrix metalloproteinases inhibitor. *Virology* 397: 130-138

97. Verma S, Lo Y, Chapagain M, Lum S, Kumar M, Gurjav U, Luo H, Nakatsuka A, and Nerurkar VR (2009) West Nile virus infection modulates human brain microvascular endothelial cells tight junction proteins and cell adhesion molecules: Transmigration across the in vitro blood-brain barrier. *Virology* 385: 425-433

98. Wang P, Dai J, Bai F, Kong KF, Wong SJ, Montgomery RR, Madri JA, and Fikrig E (2008) Matrix metalloproteinase 9 facilitates West Nile virus entry into the brain. *J Virol* 82: 8978-8985

99. Xu Z, Waeckerlin R, Urbanowski MD, van Marle G, and Hobman TC (2012) West Nile virus infection causes endocytosis of a specific subset of tight junction membrane proteins. *PLoS One* 7: e37886

100. Yamashita M, Iida M, Tada M, Shirasago Y, Fukasawa M, Nagase S, Watari A, Ishii-Watabe A, Yagi K, and Kondoh M (2015) Discovery of anti-claudin-1 antibodies as candidate therapeutics against hepatitis C virus. *J Pharmacol Exp Ther* 353: 112-118

101. Yang W, Qiu C, Biswas N, Jin J, Watkins SC, Montelaro RC, Coyne CB, and Wang T (2008) Correlation of the tight junction-like distribution of claudin-1 to the cellular tropism of hepatitis C virus. *J Biol Chem* 283: 8643-8653

102. Yeo NK, and Jang YJ (2010) Rhinovirus infection-induced alteration of tight junction and adherens junction components in human nasal epithelial cells. *Laryngoscope* 120: 346-352

103. Zeisel MB, Koutsoudakis G, Schnober EK, Haberstroh A, Blum HE, Cosset FL, Wakita T, Jaeck D, Doffoel M, Royer C, Soulier E, Schvoerer E, Schuster C, Stoll-Keller F, Bartenschlager R, Pietschmann T, Barth H, and Baumert TF (2007) Scavenger receptor class B type I is a key host factor for hepatitis C virus infection required for an entry step closely linked to CD81. *Hepatology* 46: 1722-1731

104. Zheng A, Yuan F, Li Y, Zhu F, Hou P, Li J, Song X, Ding M, and Deng H (2007) Claudin-6 and claudin-9 function as additional coreceptors for hepatitis C virus. *J Virol* 81: 12465-12471

105. Zheng J, Xie Y, Campbell R, Song J, Massachi S, Razi M, Chiu R, Berenson J, Yang OO, Chen IS, and Pang S (2005) Involvement of claudin-7 in HIV infection of CD4(-) cells. *Retrovirology* 2: 79

106. Zona L, Lupberger J, Sidahmed-Adrar N, Thumann C, Harris HJ, Barnes A, Florentin J, Tawar RG, Xiao F, Turek M, Durand SC, Duong FH, Heim MH, Cosset FL, Hirsch I, Samuel D, Brino L, Zeisel MB, Le Naour F, McKeating JA, and Baumert TF (2013) HRas signal transduction promotes hepatitis C virus cell entry by triggering assembly of the host tetraspanin receptor complex. *Cell Host Microbe* 13: 302-313